

Serial No.: 09/181,585

Confirmation No.: 1075

Filed: October 28, 1998

For: SPINOCEREBELLAR ATAXIA TYPE 8 AND METHODS OF DETECTION

- F₁ or
- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers wherein a first oligonucleotide primer of the two oligonucleotide primers is chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer of the two oligonucleotide primers is chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;
 - (b) extending the primers to form complementary primer extension products which act as templates for synthesizing the desired DNA fragment containing the repeat region;
 - (c) detecting the fragment so amplified; and
 - (d) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

2p.

(Twice Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of an SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers wherein the first oligonucleotide primer is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:4 and wherein the second oligonucleotide primer is selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:12;
 - (b) extending the primers to form complementary primer extension products which act as templates for synthesizing the desired DNA fragment containing the repeat region;
 - (c) detecting the fragment so amplified; and
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- F₁ cont
- (d) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

8. 4
- (Amended) A method for detecting the presence of at least one DNA molecule containing a repeat region of an SCA8 coding sequence comprising:
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- (a) digesting genomic DNA with a restriction endonuclease to obtain DNA fragments;
 - (b) denaturing the DNA fragments to yield DNA molecules and probing the DNA molecules under hybridizing conditions with a detectably labeled probe, which hybridizes to a DNA molecule containing a repeat region of an isolated SCA8 coding sequence, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS, wherein the probe is chosen from nucleotides 1-448 of SEQ ID NO:1 or from nucleotides 726-1,159 of SEQ ID NO:1, or complements thereto, wherein the probe has at least 20 nucleotides;
 - (c) detecting the probe which has hybridized to the DNA molecule; and
 - (d) analyzing the DNA molecule for a repeat region characteristic of a normal or at-risk form of the SCA8 coding sequence.

13. 6
- (Three Times Amended) A method for determining whether an individual is not at-risk for developing spinocerebellar ataxia type 8, the method comprising analyzing a repeat region of a spinocerebellar ataxia type 8 coding sequence wherein individuals who are not at-risk for developing spinocerebellar ataxia type 8 have less than 80 CTG repeats in
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F3 cont the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region.

18.7 (Twice Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of an SCA8 coding sequence comprising:

- F4
- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair wherein the first oligonucleotide primer pair comprises a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;
 - (b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;
 - (c) removing the first desired DNA fragment containing the repeat region;
 - (d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair;
 - (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;
 - (f) detecting the second desired DNA fragment so amplified; and
 - (g) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.
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45 11. 9 (Twice Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of an SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair;
- (b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;
- (c) removing the first desired DNA fragment containing the repeat region;
- (d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair wherein the second oligonucleotide primer pair comprises a first oligonucleotide primer chosen from nucleotides 449-725 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;
- (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;
- (f) detecting the second desired DNA fragment so amplified; and
- (g) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

33. 12 (Twice Amended) An isolated oligonucleotide consisting of at least 15 contiguous nucleotides from nucleotides 1-448 of SEQ ID NO:1, and the complementary nucleotides thereto.

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Amendment and Response Under 37 C.F.R. §1.116 - Expedited Examining Procedure

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34. ¹³ (Twice Amended) An isolated oligonucleotide consisting of at least 15 contiguous nucleotides from nucleotides 726-1,159 of SEQ ID NO:1, and the complementary nucleotides thereto.

F.W.
cont

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